

Report Investigation of Teratogenic & Toxic Potential of Methocel In Mice
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REPORT INVESTIGATION OF TERATOGENIC
AND TOXIC POTENTIAL OF METHOCCEL IN
MICE

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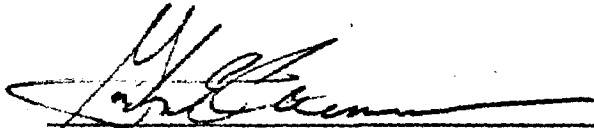
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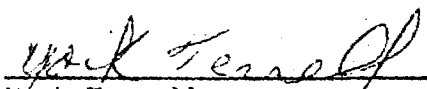
P. O. Box 3627, Reading, Pa. 19605 (215) 375-4536

REPORT

INVESTIGATION OF TERATOGENIC AND TOXIC POTENTIAL OF METHOCEL IN MICE



Dr. Gaston E. Cannon
Chief Toxicologist

Geoffrey St. E. Parke
Director of Biological Services

York Terrell
Chief Technician

John Ravert
Senior Technician

Cannon Laboratories, Inc.

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A. Detailed Animal Data Worksheets	Under Separate Cover

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This investigation was authorized by the DHEW/PUBLIC HEALTH SERVICE,
Food and Drug Administration, Rockville, Maryland, according to Government
contract number 223-74-2175.

For this test procedure, the material "Methocel FDA 71-51" was used.

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I INTRODUCTION

This report contains the results of the administration of "Methocel" to mice during gestation. The purpose of the study was to determine whether "Methocel" would induce toxic and teratogenic effects when administered orally to pregnant mice during the organogenic phase of fetal development.

II METHODS

Animals:

All animals in this study were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. Mice of the CD/1 strain were employed for these experiments.

Pretreatment:

The animals were conditioned to the laboratory environment for at least 2 weeks before the study was initiated.

Housing:

They were housed individually in wire mesh cages in air-conditioned, light and humidity controlled quarters with an artificial light cycle of 12 hours.

Diet:

Water and food (pelleted Purina Lab Chow) were freely available.

Breeding:

For mating purposes, one female was housed with one male. The females were examined for evidence of insemination, as reflected by the presence of a sperm plug on the morning following cohabitation. Following confirmation of

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insemination the animals were randomly selected and individually housed. The day of finding the sperm plug was considered as day 0 of gestation. A sufficient number of mice were mated so as to provide a minimum of 20 pregnant mice per dose level for 6 dose levels.

Dose Levels and Compound Administration:

1. The high dose was 700 mg/kg of body weight corresponding to 10% of the LD50 as a 1.2% suspension in corn oil. The volume administered was 58.33 ml/kg.
2. The low dose was 70 mg/kg of body weight corresponding to 1% of the LD50 as a 1.2% suspension in corn oil. The volume administered was 5.83 ml/kg.
3. The Intermediate level #1 was 153 mg/kg of body weight corresponding to 2.18% of the LD50 as a 1.2% suspension in corn oil. The volume administered was 12.75 ml/kg. This dosage was derived logarithmically.
4. The Intermediate level #2 was 330 mg/kg of body weight corresponding to 4.71% of the LD50 as a 1.2% suspension in corn oil. The volume administered was 27.5 ml/kg. This dosage was derived logarithmically.
5. The negative control was corn oil, administered at high dose volume.
6. The positive control was acetylsalicylic acid at 150 mg/kg, administered once daily by gavage from day 6 to day 15 of pregnancy.

Examinations Performed:

The examinations performed and data recorded in this study were of the following nature:

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Chemicals tested (or control)

Species identification

Dose level

Dam identification

Dam weights (at 0, 6, 11, 15 and 17 days)

Fate of dam (term:, died, aborted) and date

Date dam sectioned

Number of positions of implantations

Number of positions of resorptions

Number, weight and position of live fetuses

Number, weight and position of dead fetuses

Pup identification (by each dam)

Abnormalities per each pup

Sex of each pup

¹Wilson J. and Warkany J., Teratology Principles and Techniques, The University of Chicago Press, 1965.

²Refinements of Rapid Clearing Technique in KOH-Alizarin Red S Method For Fetal Bone, Stain Technology, 39 No 1, Jan. (1964).

³Wirtschafter A.T., Genesis of the Mouse Skeleton, Charles C. Thomas Publisher, Springfield, Illinois, 1960.

Cannon Laboratories, Inc.**STATISTICS:**

The statistical evaluation of the results regarding body weight of female animals, number of implantations, live fetuses, dead fetuses, resorptions and mean fetal weight was carried out for each of the six groups. The methods and results are discussed in detail in the section entitled statistical evaluation.

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III RESULTS

A. Maternal Data

1. Symptoms

The administration of "Methocel" to mice did not elicit any observable symptoms at any of the dose levels. The negative controls (given the vehicle, corn oil) and the positive control (given 150 mg/kg of acetylsalicylic acid) were also without any observable symptoms.

2. Body Weight

The mean body weight of the dams from all the dosage groups is presented in Table 1, while the individual body weight is presented in Table 2. A graphical presentation of body weight changes for pregnant animals appears in Figure 1 while another graphical presentation of all mice pregnant and nonpregnant) appears in Figure 2.

Statistical analysis revealed that there was no consistent pattern of significance among any of the groups.

3. Mortality

In the intermediate 1 level, dam no 53 died on day 15. This animal was not pregnant.

In the intermediate 2 group, dam no. 63 and 69 died on day 13, while dam no. 72 died on day 10. None of these animals were pregnant.

In the high dose, dams no. 86, 90 and 93 died on day 8, dam 83 and 98 died on day 7, and dam no. 84 died on day 9. None of these dams were pregnant.

4. Maternal Necropsy Examination

Maternal necropsy examination revealed no remarkable lesions in those animals dying during the experiment or those sacrificed at scheduled necropsy.

B. Fetal Data

Table 3 depicts the characteristics of pregnancy for each group.

Number of Implantations

Slightly higher number of mean implants in the intermediate 2 level (12.5) compared to positive control (11.8), low dose (11.3), intermediate 1 (11.3), negative control (11.2) and low dose (10.5).

Number of Live Fetuses

Slightly higher percentage in intermediate 1 group (95.8) compared to low dose (92.4), intermediate 2 (92.0), high dose (90.4), positive control (90.0) and negative control (86.3).

Number of Dead Fetuses

Slightly higher percentage in the positive control (5.0) compared to negative control (2.1), high dose (1.4), low and intermediate 1 (1.0), and intermediate 2 (0.0).

Number of Resorptions

The percentage of resorptions was slightly higher in the negative control (11.5) compared to high dose (8.0), intermediate 2 (7.9), low dose (7.0), positive control (5.0), and intermediate 1 (3.0).

Fetal Weight

The mean fetal weight ranged from 0.9 to 1.0 grams.

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Fetal Examination

Data obtained from the various methods of fetal examination are summarized in the external, visceral and skeletal observation tabulations (Table 5 external, Table 6 visceral and Table 7 skeletal).

1. External Examinations

Exencephaly, umbilical hernia, and club foot were the only external abnormalities observed.

In the negative control, one pup from dam no. 9 exhibited exencephaly.

Umbilical hernia was observed in one pup from dam no. 13 of the negative control and one pup from dam no. 27 of the low dose group.

Club foot was observed in one pup from dam no. 87 of the high dose group.

2. Visceral Examination (Wilson Technique)

Hydrocephalus, cleft palate, ectopic kidney and hydronephrosis were the principal abnormalities observed and these occurred to a very limited extent as shown in Table 6.

3. Skeletal Examination (Alizarin Technique)

Table 7 shows group incidences of various observations considered to be deviations from anticipated general fetal skeletal morphology. Pups with 14th rib bud and thirteen pairs of ribs comprised the most common deviations from normal skeletal morphology.

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IV SUMMARY AND CONCLUSION

"Methocel" was tested for its teratogenic potential and toxic effects on female mice. Pregnant animals were administered "Methocel" from days 6 to 15 of gestation. "Methocel" was administered according to the following dosage schedule.

High - 700 mg/kg

Low - 70 mg/kg

Intermediate 1 - 153 mg/kg

Intermediate 2 - 330 mg/kg

In addition, a positive control group was administered 150 mg/kg of acetylsalicylic acid. A further control group was given the suspension agent corn oil. On day 17 of gestation all surviving animals were sacrificed and submitted to a caesarean section wherein the urogenital tracts and reproductive capabilities were examined and evaluated. Parameters measured included body weight of dams at days 0, 6, 11, 15 and 17.

Number of Implantations

Number of Live Fetuses

Number of Corpora Lutea

Number of Dead Fetuses

Number of Resorptions

Weight of Fetuses

In addition, pups were evaluated for abnormalities by external, visceral and skeletal examination.

The results indicate that the test material did not induce any adverse responses with respect to survival, appearance or behavior of these animals.

External gross examination revealed exencephaly, umbilical hernia and club foot as the deviations from normal. These deviations occurred to a very limited extent. Exencephaly was confined to a single incidence in the negative control. Umbilical hernia was an abnormality affecting one pup from the negative control and one pup from the low dose group.

The Wilson Technique revealed hydrocephalus, cleft palate, ectopic kidney and hydronephrosis. These occurred only to a limited extent.

The skeletal deviations were mainly thirteen pairs of ribs and 14th rib bud.

The results obtained in this study demonstrate that "Methocel" did not induce changes consistent with criteria indicative of teratogenic or toxic activity.

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TABLE 1: MEAN BODY WEIGHT OF MICE ADMINISTERED METHOCEL

DOSE LEVEL	MG/KG	DAYS					CHANGE IN BODY WT.
		0	6	11	15	17	
A							
Neg. Control	Vehicle	26.2	28.8	32.9	37.7	45.9	19.7
1/100 LD50	70.00	25.5	27.7	31.1	36.6	41.3	15.8
Intermediate 1	153.00	26.0	27.1	30.6	39.9	47.4	21.2
Intermediate 2	330.00	26.2	27.9	30.8	40.1	47.9	21.7
1/10 LD50	700.00	26.6	27.5	32.2	38.4	44.9	18.3
Positive Control	150.00 Acetylsalicylic Acid	26.9	28.5	31.4	37.8	47.2	20.3
B							
Neg. Control	Vehicle	26.5	29.5	34.2	40.3	49.2	22.7
1/100 LD50	70.00	25.6	28.6	33.0	40.6	46.3	20.7
Intermediate 1	153.00	26.1	27.5	31.3	41.6	49.9	23.8
Intermediate 2	330.00	26.4	28.4	31.8	42.3	50.8	24.4
1/10 LD50	700.00	26.9	28.1	30.6	40.5	47.9	21.0
Positive Control	150.00 Acetylsalicylic Acid	27.0	28.9	32.4	39.7	50.8	23.8

A - Body weight of all animals

B - Body weight not including aborted, dead and non-pregnant animals

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INDIVIDUAL BODY WEIGHTS OF MICE DAMS ADMINISTERED METHOCEL

DOSE LEVEL	ANIMAL NUMBER	DAY				
		0	6	11	15	17
Negative Control Vehicle mg/kg	1	26	30	34	45	52
	2	28	34	39	35	52
	3	28	33	31	34	50
	4	29	32	32	31	47
	5	26	32	32	32	47
	6	27	29	28	45	52
	7	26	26	25	38	42
	8	27	25	35	43	54
	9	28	31	39	46	57
	*10	25	20	22	20	26
	11	29	31	40	47	50
	12	26	30	40	45	52
	13	27	29	43	41	46
	14	22	26	32	40	46
	15	29	32	35	49	50
	16	23	28	33	37	47
	17	24	25	30	37	48
	*18	22	26	26	23	27
	19	26	29	33	40	44
	*20	26	28	29	26	28

* NOT PREGNANT

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INDIVIDUAL BODY WEIGHTS OF MICE DAMS ADMINISTERED METHOCEL

DOSE LEVEL	ANIMAL NUMBER	DAY				
		0	6	11	15	17
1/100 LD50 70.00 mg/kg	21	23	26	31	33	42
	*22	24	26	26	23	27
	23	25	30	35	40	46
	*24	26	19	19	18	18
	25	26	28	32	37	43
	26	28	30	35	48	50
	27	26	28	32	42	48
	28	25	30	34	42	48
	29	27	29	33	43	50
	30	25	28	31	37	43
	31	27	29	34	40	47
	32	26	32	37	46	52
	*33	26	28	28	28	29
	34	27	27	32	36	38
	35	25	27	28	38	43
	36	25	29	34	41	47
	*37	26	29	29	28	30
	38	25	27	33	43	49
	39	24	29	34	43	49
	*40	24	24	24	25	26

* NOT PREGNANT

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INDIVIDUAL BODY WEIGHTS OF MICE DAMS ADMINISTERED METHOCEL

DOSE LEVEL	ANIMAL NUMBER	DAY				
		0	6	11	15	17
Intermediate 1 153.00 mg/kg	41	27	30	35	44	54
	*42	26	27	26	25	24
	43	26	28	32	41	46
	*44	24	24	25	26	28
	45	28	30	36	46	54
	46	24	25	33	43	52
	47	26	27	34	43	48
	48	26	29	35	44	50
	49	26	29	34	46	51
	50	24	26	33	43	49
	51	26	28	35	51	51
	52	26	28	33	39	46
	53	26	25	28	DNP	DNP
	54	27	28	30	39	49
	55	27	26	28	35	45
	56	26	26	27	39	53
	57	26	25	25	40	51
	58	26	28	28	38	48
	59	27	27	28	40	55
	60	26	27	26	36	47

* NOT PREGNANT

DNP = DIED, NOT PREGNANT

TABLE 2

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Cannon Laboratories, Inc.INDIVIDUAL BODY WEIGHTS OF MICE DAMS
ADMINISTERED METHOCEL

DOSE LEVEL	ANIMAL NUMBER	DAY				
		0	6	11	15	17
Intermediate 2 330.00 mg/kg	61	28	29	32	43	55
	62	27	25	27	44	50
	63	25	25	27	DNP	DNP
	64	27	29	28	35	52
	65	26	26	29	39	48
	66	26	25	28	43	50
	*67	24	23	19	21	26
	68	26	28	28	43	49
	69	26	27	29	DNP	DNP
	70	30	31	33	46	56
	71	26	28	27	38	49
	72	28	28	DNP	DNP	DNP
	73	28	34	39	47	55
	74	27	31	36	45	52
	75	26	28	32	40	49
	76	23	26	32	40	48
	77	25	28	34	41	46
	**78	25	28	34	26	27
	79	26	30	37	45	52
	80	26	28	35	46	51

* NOT PREGNANT

** - DELIVERED BEFORE TERM

DNP = DIED, NOT PREGNANT

TABLE 2

Cannon Laboratories, Inc.¹⁸

INDIVIDUAL BODY WEIGHTS OF MICE DAMS ADMINISTERED METHOCEL

DOSE LEVEL	ANIMAL NUMBER	DAY				
		0	6	11	15	17
1/10 LD50 700.00 mg/kg	*81	27	27	27	25	28
	82	24	27	33	42	50
	83	20	25	DNP	DNP	DNP
	84	26	27	DNP	DNP	DNP
	85	30	31	36	45	53
	86	28	26	DNP	DNP	DNP
	87	25	28	35	40	55
	88	27	27	29	30	32
	89	27	25	32	44	51
	90	28	29	DNP	DNP	DNP
	91	28	27	33	40	45
	92	25	28	33	38	42
	93	25	25	DNP	DNP	DNP
	94	29	30	38	43	53
	95	27	27	33	42	47
	96	28	30	35	42	50
	*97	27	24	25	27	26
	98	29	30	DNP	DNP	DNP
	99	25	27	29	38	43
	100	28	31	34	42	54

* NOT PREGNANT

DNP = DIED, NOT PREGNANT

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INDIVIDUAL BODY WEIGHTS OF MICE DAMS ADMINISTERED METHOCEL

DOSE LEVEL	ANIMAL NUMBER	DAY				
		0	6	11	15	17
Positive Control	101	26	29	32	40	51
150.00 mg/kg	102	26	28	30	37	45
Acetylsalicylic Acid	103	28	28	33	40	56
	*104	26	23	23	25	24
	105	26	28	33	41	53
	106	26	31	34	40	50
	107	28	31	32	39	59
	108	26	28	30	36	46
	109	28	30	32	40	51
	110	28	30	36	42	56
	111	28	27	32	40	50
	*112	26	28	31	29	28
	*113	27	28	24	26	28
	114	27	27	30	37	48
	115	27	28	34	41	48
	116	27	29	30	43	47
	117	28	31	34	39	53
	118	27	29	36	42	52
	119	27	29	31	39	48
	120	26	28	31	39	50

* NOT PREGNANT

Pregnant Mice - Methocel

Body Weight Change

FIGURE 1: AVERAGE CHANGE IN BODY WEIGHT
(AT 6, 11, 15 AND 17 DAYS)

PREGNANT MICE

GMS

30

25

20

15

10

5

0

DAYS

0

6

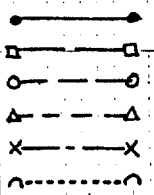
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15

17

INT 1
Pos. Control
INT 2
Neg. Control
1/10 LD50
1/100-LD50

Symbols
Negative Control
Positive Control
1/100 LD50
INT 1
INT 2
1/10 LD50



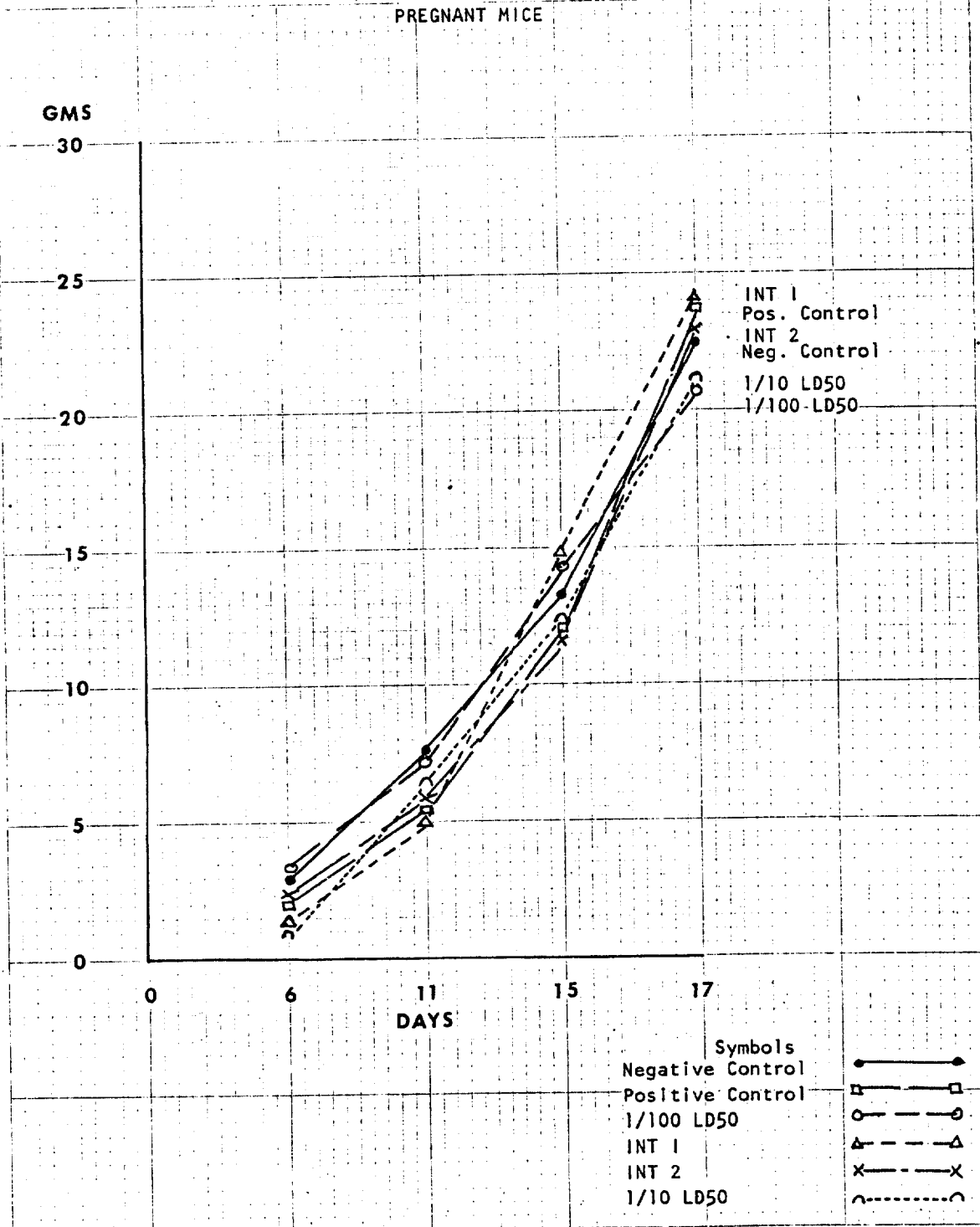
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10 X 10 TO THE INCH • 7 X 10 INCHES
KEUFFEL & ESSER CO. MADE IN U.S.A.

Pregnant Mice - Methocel

Body Weight Change

FIGURE 1: AVERAGE CHANGE IN BODY WEIGHT
(AT 6, 11, 15 AND 17 DAYS)
PREGNANT MICE



46 0700

10 X 10 TO THE INCH, 7 X 10 INCHES
KEUFFEL & ESSER CO. MADE IN U.S.A.

All Mice - Methocel

Body Weight Change

FIGURE 2: AVERAGE BODY WEIGHT CHANGE
(AT 6, 11, 15 AND 17 DAYS)
ALL MICE

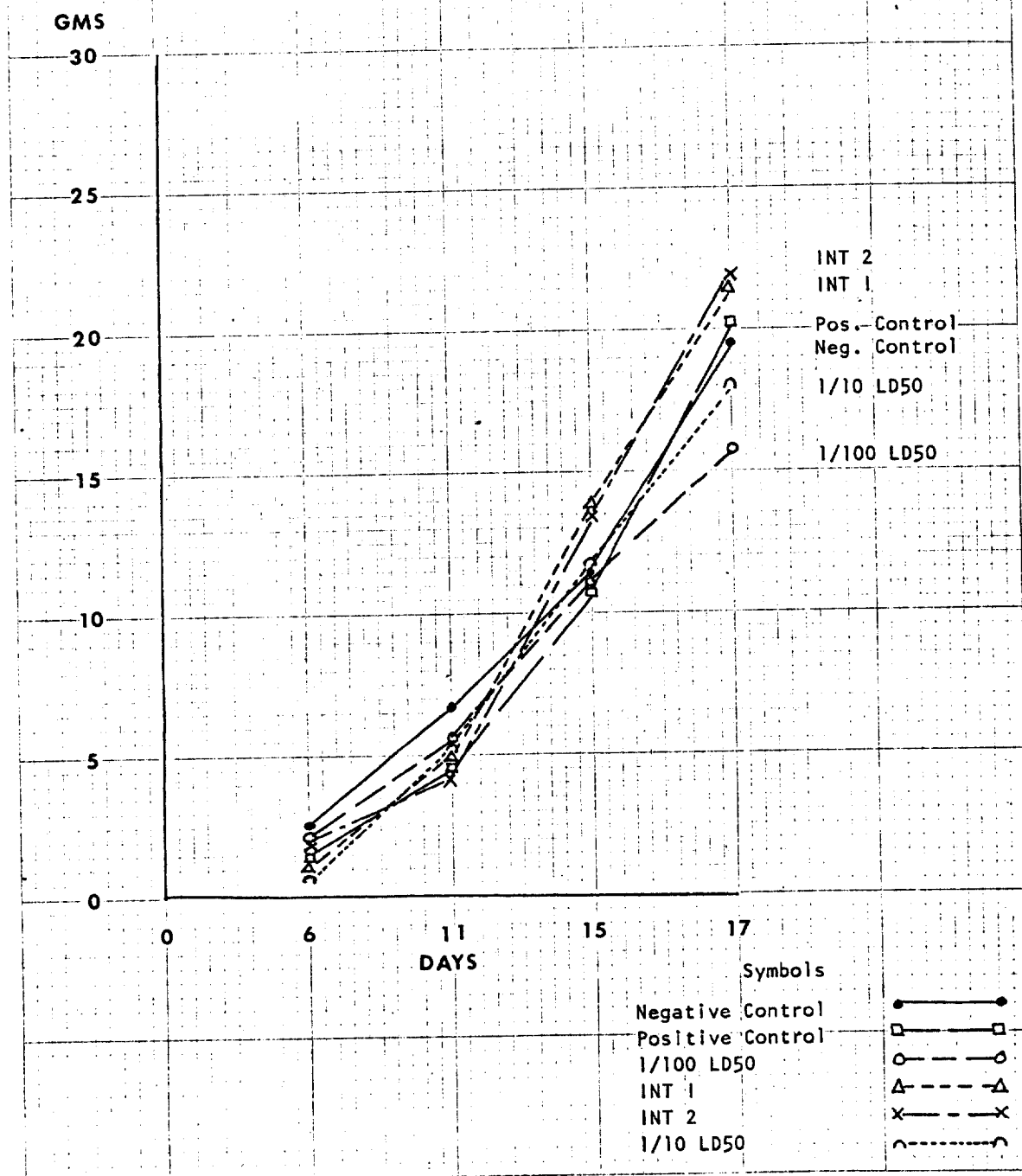


TABLE 3: SUMMARY DATA; TERATOLOGY STUDY IN MICE; METHOCEL

GROUP CHARACTERISTICS OF PREGNANCY

DOSE LEVEL	MG/KG	MICE EXHIBITING PLUGS	MICE PREGNANT	IMPLANT (MEAN)	LIVE FETUS (%)	DEAD FETUS (%)	RESORPTIONS (%)	CORPORA LUTEA MEAN	MEAN FETAL WT.
Neg. Control	Vehicle	20	17	191 (11.2)	165 (86.39)	4 (2.1)	22 (11.5)	205 (12.1)	1.0
1/100 LD50	70.00	20	15	157 (10.5)	145 (92.4)	1 (1)	11 (7.0)	281 (18.7)	1.0
Intermediate 1	153.00	20	17	192 (11.3)	184 (95.8)	2 (1.0)	6 (3.0)	275 (16.2)	1.0
Intermediate 2	330.00	20	15	188 (12.5)	173 (92.0)	0 (0)	15 (7.9)	223 (14.9)	1.0
1/10 LD50	700.00	20	12	136 (11.3)	123 (90.4)	2 (1.4)	11 (8)	173 (14.4)	0.9
Positive Control	150.00 Acetylsalicylic Acid	20	17	200 (11.8)	180 (90.0)	10 (5)	10 (5)	213 (12.5)	1.0

TABLE 4

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TERATOLOGY STUDY IN MICE: METHOCEL
INDIVIDUAL DATA
NEGATIVE CONTROL VEHICLE MG/KG

DAM NUMBER	IMPLANTS	CORPORA LUTEA	VIABLE FETUSES	NON-VIABLE FETUSES	RESORPTIONS	AVERAGE FETAL WGT. (GMS)
1	14	15	13	0	1	.9
2	11	12	7	0	4	1.1
3	11	12	8	0	3	1.5
4	13	13	11	1	1	.9
	11	11	11	0	0	1.0
6	14	14	13	1	0	.9
7	11	12	9	0	2	.6
8	12	14	11	0	1	1.2
9	11	11	11	0	0	1.3
10	NP	NP	NP	NP	NP	NP
11	12	12	9	0	3	1.0
12	12	15	11	0	1	1.2
13	10	12	8	1	1	.9
14	10	10	8	1	1	1.0
15	12	12	11	0	1	.9
16	9	12	8	0	1	1.1
	12	12	11	0	1	1.1
18	NP	NP	NP	NP	NP	NP
19	6	6	5	0	1	1.2
20	NP	NP	NP	NP	NP	
TOTAL NUMBER	191	205	165	4	22	
AVERAGE	11.2	12.1	9.7	< 1	> 1	1.0

NP = NOT PREGNANT

TABLE 4

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TERATOLOGY STUDY IN MICE: METHOCEL
 INDIVIDUAL DATA
 1/100 LD50 70.00 MG/KG

DAM NUMBER	IMPLANTS	CORPORA LUTEA	VIABLE FETUSES	NON-VIABLE FETUSES	RESORPTIONS	AVERAGE FETAL WGT. (GMS)
21	9	9	9	0	0	.9
22	NP	NP	NP	NP	NP	NP
23	11	22	10	0	1	1.0
24	NP	NP	NP	NP	NP	NP
25	10	21	10	0	0	1.0
26	11	19	11	0	0	1.0
27	10	17	10	0	0	1.1
28	11	22	11	0	0	1.1
29	12	20	12	0	0	.9
30	10	18	8	0	2	.9
31	10	19	8	0	2	1.1
32	13	20	11	1	1	1.0
33	NP	NP	NP	NP	NP	NP
34	5	13	2	0	3	1.1
35	10	20	9	0	1	1.0
36	10	24	10	0	0	1.0
37	NP	NP	NP	NP	NP	NP
38	11	17	11	0	0	1.1
39	14	20	13	0	1	1.0
40	NP	NP	NP	NP	NP	NP
TOTAL NUMBER	157	281	145	1	11	
AVERAGE	10.5	18.7	9.7	<1	<1	1.0

NP = NOT PREGNANT

TABLE 4

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TERATOLOGY STUDY IN MICE: METHOCEL
INDIVIDUAL DATA
INTERMEDIATE I 153.00 MG/KG

DAM NUMBER	IMPLANTS	CORPORA LUTEA	VIABLE FETUSES	NON-VIABLE FETUSES	RESORPTIONS	AVERAGE FETAL WGT. (GMS)
41	12	19	12	0	0	1.0
42	NP	NP	NP	NP	NP	NP
43	11	18	11	0	0	1.0
44	NP	NP	NP	NP	NP	NP
45	13	18	13	0	0	1.1
46	13	20	12	0	1	1.1
47	11	15	11	0	0	1.1
48	10	16	9	1	0	1.2
49	11	17	10	1	0	0.9
50	11	17	11	0	0	1.1
51	8	13	7	0	1	1.1
52	12	20	12	0	0	1.1
53	DNP	DNP	DNP	DNP	DNP	DNP
54	12	18	12	0	0	1.0
55	13	17	12	0	1	1.1
56	11	15	11	0	0	1.0
57	11	13	9	0	2	0.9
58	10	11	10	0	0	0.8
59	13	15	12	0	1	0.9
60	10	13	10	0	0	1.0
TOTAL NUMBER	192	275	184	2	6	
AVERAGE	11.3	16.2	10.8	< 1	< 1	1.0

NP = NOT PREGNANT

DNP = DIED, NOT PREGNANT

TABLE 4

26
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TERATOLOGY STUDY IN MICE: METHOCEL
 INDIVIDUAL DATA
 INTERMEDIATE 2 330.00 MG/KG

DAM NUMBER	IMPLANTS	CORPORA LUTEA	VIALE FETUSES	NON-VIALE FETUSES	RESORPTIONS	AVERAGE FETAL WGT. (GMS)
61	10	15	10	0	0	1.5
62	11	17	11	0	0	1.2
63	DNP	DNP	DNP	DNP	DNP	DNP
64	14	18	11	0	3	1.0
65	11	14	11	0	0	0.8
66	12	16	11	0	1	0.9
67	NP	NP	NP	NP	NP	NP
68	13	15	13	0	0	0.9
69	DNP	DNP	DNP	DNP	DNP	DNP
70	12	13	11	0	1	1.4
71	12	14	12	0	0	1.0
72	DNP	DNP	DNP	DNP	DNP	DNP
73	16	18	14	0	2	0.9
74	16	17	13	0	3	0.8
75	15	15	13	0	2	0.7
7	12	12	11	0	1	0.9
77	12	15	12	0	0	0.8
78	DBT	DBT	DBT	DBT	DBT	DBT
79	10	10	9	0	1	1.4
80	12	14	11	0	1	1.0
TOTAL NUMBER	188	223	173	0	15	
AVERAGE	12.5	14.9	11.5	0	1.0	1.0

NP = NOT PREGNANT

DNP = DIED, NOT PREGNANT

DBT = DELIVERED BEFORE TERM

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TERATOLOGY STUDY IN MICE: METHOCEL
INDIVIDUAL DATA
1/10 LD50 700.00 MG/KG

DAM NUMBER	IMPLANTS	CORPORA LUTEA	VIABLE FETUSES	NON-VIABLE FETUSES	RESORPTIONS	AVERAGE FETAL WGT. (GMS)
81	NP	NP	NP	NP	NP	NP
82	11	11	10	0	1	1.0
83	DNP	DNP	DNP	DNP	DNP	DNP
84	DNP	DNP	DNP	DNP	DNP	DNP
85	13	14	12	0	1	.9
86	DNP	DNP	DNP	DNP	DNP	DNP
87	12	15	11	1	0	1.2
88	1	18	1	0	0	.7
89	13	14	13	0	0	1.0
90	DNP	DNP	DNP	DNP	DNP	DNP
91	12	14	9	1	2	.8
92	12	12	10	0	2	.8
93	DNP	DNP	DNP	DNP	DNP	DNP
94	14	16	13	0	1	.9
95	11	16	9	0	2	1.0
96	13	14	13	0	0	.9
97	NP	NP	NP	NP	NP	NP
98	DNP	DNP	DNP	DNP	DNP	DNP
99	10	12	9	0	1	.9
100	14	17	13	0	1	1.0
TOTAL NUMBER	136	173	123	2	11	
AVERAGE	11.3	14.4	10.3	<1	<1	.9

NP = NOT PREGNANT

DNP = DIED, NOT PREGNANT

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TERATOLOGY STUDY IN MICE: METHOCEL
 INDIVIDUAL DATA
 POSITIVE CONTROL 150.00 MG/KG ACETYLSALICYLIC ACID

DAM NUMBER	IMPLANTS	CORPORA LUTEA	VIABLE FETUSES	NON-VIABLE FETUSES	RESORPTIONS	AVERAGE FETAL WGT. (GMS)
101	8	11	6	1	1	1.1
102	12	16	10	1	1	1.1
103	13	16	11	2	0	1.2
104	NP	NP	NP	NP	NP	NP
105	11	11	9	1	1	1.2
106	12	13	11	0	1	.9
107	13	13	13	0	0	.9
108	12	13	11	0	1	.9
109	12	12	11	0	1	.9
110	13	14	13	0	0	1.2
111	12	12	11	1	0	1.0
112	NP	NP	NP	NP	NP	NP
113	NP	NP	NP	NP	NP	NP
114	11	11	10	1	0	.9
115	12	12	12	0	0	1.1
116	13	13	10	0	3	.8
117	14	14	12	2	0	.7
118	10	10	9	0	1	1.1
119	11	11	11	0	0	1.2
120	11	11	10	1	0	.9
TOTAL NUMBER	200	213	180	10	10	
AVERAGE	11.8	12.5	10.6	<1	<1	1.0

NP = NOT PREGNANT

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TABLE 5: GROUP INCIDENCE OF FETAL OBSERVATIONS IN MICE
EXTERNAL EXAMINATION

COMPOUND: METHOCEL

	DOSE LEVELS					
	NEG. CONTROL	1/100 LD50	INT I	INT II	1/10 LD50	POSITIVE CONTROL
NUMBER OF FETUSES EXAMINED	169	146	186	173	125	190
EXT. ABNORMALITIES						
Exencephaly	1	0	0	0	0	0
Encephaly	0	0	0	0	0	0
Cleft palate	0	0	0	0	0	0
Cleft lip	0	0	0	0	0	0
Abnormal Ear	0	0	0	0	0	0
Snout repressed	0	0	0	0	0	0
Retarded mandibles	0	0	0	0	0	0
Micromelia	0	0	0	0	0	0
Umbilical hernia	1	1	0	0	0	0
Club foot	0	0	0	0	1	0
Polydactylous fore paw	0	0	0	0	0	0
Polydactylous rear paw	0	0	0	0	0	0
Ectrodactylous fore paw	0	0	0	0	0	0
Ectrodactylous rear paw	0	0	0	0	0	0
HEMATOMA						
(a) Cephalic	0	0	0	0	0	0
(b) Thoracic	0	0	0	0	0	0
(c) Abdominal	0	0	0	0	0	0
Tail Defect	0	0	0	0	0	0
OTHER	0	0	0	0	0	0

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TABLE 6: GROUP SUMMARY OF WILSON SECTION FINDINGS

COMPOUND: METHOCEL

SPECIE: MICE

	DOSE LEVELS					POSITIVE CONTROL
	NEG. CONTROL	1/100 LD50	INT I	INT II	1/10 LD50	
NUMBER OF FETUSES EXAMINED	53	43	58	53	43	59

HEAD:

Hydrocephalus	1	0	1	0	0	0
Micrencephalus	0	0	0	0	0	0
Anophthalmia	0	0	0	0	0	0
Exophthalmia	0	0	0	0	0	0
Nasal Septum Incomplete	0	0	0	0	0	0
Cleft Palate	1	0	0	1	1	0
Harelip	0	0	0	0	0	0
Small Mandible	0	0	0	0	0	0

TRUNK:

Missing esophagus	0	0	0	0	0	0
Tracheo-esophageal Fistula	0	0	0	0	0	0
Apulmonism	0	0	0	0	0	0
Reverse Dorsal Aorta	0	0	0	0	0	0
Atrial Septum Incomplete	0	0	0	0	0	0
Ventricular Septum Incomplete	0	0	0	0	0	0
Diaphragmatic Hernia	0	0	0	0	0	0
Etopic Kidney	0	0	0	1	0	0
Fused Kidneys	0	0	0	0	0	0
Hydronephrosis	0	0	0	1	0	1
Enlarged Renal Pelvis	0	0	0	0	0	0
Testes Incomplete Descending	0	0	0	0	0	0

OTHER

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GROUP SUMMARY OF OBSERVATIONS IN ALIZARIN STAINED FETUSES

COMPOUND: METHOCEL

SPECIE: MICE

	DOSE LEVELS					
	NEG. CONTROL	1/100 LD50	INT I	INT II	1/10 LD50	POSITIVE CONTROL
NUMBER OF FETUSES EXAMINED	114	100	128	120	86	134
ABNORMALITIES						
STERNEBRAE:						
Incomplete Ossification	0	0	0	0	0	0
Scrambled	0	0	0	0	0	0
Bipartite	0	0	0	0	0	0
Fus	0	0	0	0	0	0
Extra	0	0	0	0	0	0
Missing	0	0	0	0	0	0
Malaligned	0	0	0	0	0	0
RIBS:						
Incomplete Ossification	0	0	0	0	0	0
Fused/Split	0	0	0	0	0	0
Wavy	0	2	0	0	0	0
Less than 12	0	0	0	0	0	0
More than 13	23	10	22	15	21	11
14th Bud	15	13	23	26	25	19
VERTEBRAE:						
Incomplete Ossification	0	0	0	0	0	0
Scrambled	0	0	0	0	0	0
Fus	0	0	0	0	0	0
Extra Center Oss	0	0	0	0	0	0
Scoliosis	0	0	0	0	0	0
Tail defects	0	0	0	0	0	0
SKULL:						
A. Nonossification of -						
1. Parietals	0	0	0	0	2	0
2. Occipitals	0	0	0	0	2	0
3. Frontals	0	0	0	0	2	0
B. Retarded Nasal Development	0	0	0	0	0	3
C. Wide Bone Separation in Skull	0	0	0	0	0	0
OTHER:	0	0	0	0	0	0



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VI STATISTICAL EVALUATION

**Cannon Laboratories, Inc.**STATISTICAL ANALYSIS

RESULTS OF EXPERIMENT WITH METHOCEL

IN MICE

1.0 General

1.1 A statistical analysis was performed on the experimentation results involving "Methocel" in mice. This analysis covered the following

1.1.1 Changes in body weight from:

- a) 0-6 days
- b) 0-11 days
- c) 0-15 days
- d) 0-17 days

1.1.2 Other teratology results:

- a) Implants
- b) Corpora Lutea
- c) Viable Fetuses
- d) Nonviable Fetuses
- e) Resorptions
- f) Average Fetal Weight

1.2 The report is divided into two parts:

1.2.1 Part I: Explanations of Statistical Methods Used.

1.2.2 Part II: Conclusions From Statistical Analysis.

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PART I

STATISTICAL METHODS

1.0 Comparisons were made among the results of the six experimental groups at each time interval for statistical significance. The following recognized standard statistical methods were used for testing for any significances at 95% or 99% confidence levels. The sequence of testing the data follows the methods listed below:

1.1 Bartlett's Test For Homogeneity of Variances:

The results of this test determined which statistical test could be validly applied to compare the means (averages) of the six experimental groups:

1.1.1 If the experimental group variances demonstrated homogeneity, the Analysis of Variances was used to determine group or groups significances or nonsignificances.

1.1.2 If the variances demonstrated heterogeneity, Wilcoxon's Rank Sum test was used for group comparisons.

1.2 Analysis of Variance

1.2.1 If significant group differences were found by means of this test, Duncan's Multiple Range Test was applied to determine which mean or means was significantly higher or lower.



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1.3 Duncan's Multiple Range Test

1.3.1 This test determines which mean or means is significantly higher or lower.

1.4 Wilcoxon's Rank Sum Test

1.4.1 This test determines which mean or means is significantly higher or lower.

2.0 Attached Summary Tables

2.1 Each table presents the following results for each statistical test at each time period:

2.1.1 Significant Differences - Yes or No

If "Yes" the confidence level is given.

2.1.2 F-Ratio or Bartlett's Ratio - These ratios are calculated from the data and are compared to the significant value of the F-Statistic or Bartlett's Statistic.

2.1.3 Means and sigmas of the change for each experimental group.

2.2 The test used to determine significance of means (Duncan's Multiple Range or Wilcoxon's Rank Sum) and the conclusions from these tests.

PART II

DETAILED CONCLUSIONS

The statistical tests, which are described in Part I, were used to determine significant differences among the data of the six experimental groups at each time period. The following conclusions will present any significant differences found with accompanying results of the test or tests used to find the significance, the statistical tests used (described in Part I), obtained statistics or results of significance comparisons, and the means and sigmas of each experimental group.

Each data summary sheet presents the following information.

- A. Parameter Being Tested: Changes in body weight, and other teratology results.
- B. Time Period: 6, 11, 15, and 17 days.
- C. Analysis of Variance Results:

F-Ratio: A calculated value from the data.

F-Statistic: This is the limiting value obtained from the statistical F-Table from the quantity of data used.

NOTE: If this calculated F-Ratio exceeds the F-Statistic, there is a significant difference between the experimental groups.

Significant Difference and Confidence Level: A significant difference and confidence level in the result is denoted by "Yes @ .05" (95% confidence level) or "Yes @ .01" (99% confidence level).



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D. Bartlett's Test Results:

Bartlett Ratio: A calculated value from the data.

Bartlett Statistic: This is the limiting value of this test.

NOTE: If the calculated ratio exceeds the statistic, heterogeneity exists between the group variances, hence the analysis of variance cannot be used to test the group means for significant differences.

Significant Difference and Confidence Level: A significant difference and confidence level in the results is denoted by either "Yes @ .05" or "Yes @ .01".

E. Means and Sigmas (Standard Deviations) of the Six Experimental Groups.

F. Concluding Statement of Tests: Underneath the above information (A through E) is a statement of which mean or means is significantly less or greater than others and at which confidence level. The statistical test used to determine the significance is also given.

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PART II

CONCLUSIONS SUMMARY

1.0 Methocel

1.1 Pregnant Mice: Various significant differences were found for body weight change, but no outstanding pattern was determined.

The means for each group are as follows:

TIME PERIOD	EXPERIMENTAL GROUPS					
	NEG. CONT.	POS. CONT.	1/100 LD50	INT. 1	INT. 2	1/10 LD50
6 Days	3.0	1.9	3.0	1.4	2.0	1.2
11 Days	7.6	5.4	7.4	5.2	5.6	6.4
15 Days	13.2	12.1	14.3	14.9	11.8	11.9
17 Days	22.6	23.8	20.7	23.8	22.9	21.0

A chart of these results and a detailed statistical data sheet are included on the following pages.

1.1.1 Significant differences were found for:

- a. Resorptions: Negative control group significantly higher than other groups.
- b. Corpora Lutea: 1/100 LD50 group significantly higher than other groups.

The means for each group are as follows:

	EXPERIMENTAL GROUPS					
	NEG. CONT.	POS. CONT.	1/100 LD50	INT. 1	INT. 2	1/10 LD50
Implants	11.2	11.8	10.5	11.3	12.5	11.3
Cor. Lut.	12.1	12.5	18.9	16.2	14.9	14.4
Via. Fet.	9.7	10.6	9.7	10.8	11.5	10.2
Nonvia. Fet.	0.23	0.59	0.06	0.12	0	0.17
Resorp.	1.29	0.59	0.73	0.35	1.00	0.92
Av. Fet. Wt.	1.05	1.01	1.01	1.50	1.01	0.92

A detailed statistical data sheet of these results is included on the following page:

1.2 All Mice (pregnant and nonpregnant, included)

A significant difference was found for body weight change at 6 days, but no outstanding pattern was determined. The means for each group are as follows:

TIME PERIOD	EXPERIMENTAL GROUPS					
	NEG. CONT.	POS. CONT.	1/100 LD50	INT. 1	INT. 2	1/10 LD50
6 Days	2.6	1.6	2.2	1.2	1.6	0.8
11 Days	6.7	4.5	5.6	4.6	4.7	5.4
15 Days	11.5	10.8	11.1	13.9	13.8	11.5
17 Days	19.6	20.2	15.8	21.4	21.7	18.0

A chart of these results and a detailed statistical data sheet are included on the following pages.

EXP. 3083
METHOCEL

CHANGE IN BODY WEIGHT - MICE - ALL MICE

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TIME PERIOD	ANALYSIS OF VARIANCE			BARTLETT'S TEST			EXPERIMENTAL GROUPS (MEAN AND σ)					
	F-RATIO	F-STAT.	SIG. DIFF. AND CONF. LEVEL	BARTLETT RATIO	BARTLETT STAT.	SIG. DIFF. AND CONF. LEVEL	MEAN AND SIGMAS	NEG. CONTROL	POS. CONTROL	1/100 LD 50	INT. 1	INT. 2 LD
0-6 Days	2.01	2.30	No	13.18	11.1	Yes	Mean	2.6	1.6	2.2	1.2	1.6
			Wilcoxon's Test Results: Negative Control and 1/100 LD 50 groups significantly greater weight change than other 4 groups at .05 confidence level.			0.01	σ	2.6	1.7	2.7	1.3	2.0
0-11 Days	0.87	2.30	No	3.56	11.1	No	Mean	6.7	4.5	5.6	4.6	4.7
			No significant difference at .05 confidence level.				σ	4.9	3.1	4.2	3.8	4.4
0-15 Days	0.82	2.30	No	4.22	11.1	No	Mean	11.5	10.8	11.1	13.9	13.9
			No significant difference at .05 confidence level.				σ	7.5	4.9	7.9	6.2	6.4
0-17 Days	1.26	2.30	No	2.44	11.1	No	Mean	19.6	20.2	15.8	21.4	21.7
			No significant difference at .05 confidence level.				σ	8.0	9.1	9.7	7.8	7.6

EXP. 3083

CHANGE IN BODY WEIGHT - MICE - PREGNANT MICE

CANNON LABORATORIES, INC.

METHOCEL

TIME PERIOD	ANALYSIS OF VARIANCE			BARTLETT'S TEST			EXPERIMENTAL GROUPS (MEAN AND σ)					
	F-RATIO	F-STAT.	SIG. DIFF. AND CONF. LEVEL	BARTLETT RATIO	BARTLETT STAT.	SIG. DIFF. AND CONF. LEVEL	MEAN AND SIGMAS	NEG. CONTROL	POS. CONTROL	1/100 LD 50	INT. 1	INT. 2
0-6 Days	3.12	2.30	Yes @.05	6.02	11.1	No	Mean \sim	3.0 2.1	1.9 1.4	3.0 1.6	1.4 1.3	2.0 2.0
<u>Duncan's Test Results:</u> Int. 1 and 1/10 LD 50 groups significant weight change less than other 4 groups at .05 confidence level.												
0-11 Days	1.72	2.30	No	15.43	15.1	Yes @.01	Mean \sim	7.6 4.5	5.4 1.9	7.4 2.2	5.2 3.8	5.6 3.9
<u>Wilcoxon's Test Results:</u> Int. 1 group significantly less weight change than negative control and 1/10 LD 50 groups at .01 confidence level.												
0-15 Days	1.34	2.30	No	9.41	11.1	No	Mean \sim	13.2 5.4	12.1 2.8	14.3 3.7	14.9 4.4	11.8 5.6
No significant difference at .05 confidence level.												
0-17 Days	1.38	2.30	No	26.64	15.1	Yes @.01	Mean \sim	22.6 3.6	23.8 3.4	20.7 3.8	23.8 9.4	22.9 5.8

Wilcoxon's Test Results:

Positive control and Int. 1 groups significantly greater weight change than 1/10 LD 50 and 1/100 LD 50 groups at .05 confidence level.

METHOCEL

CANNON LABORATORIES, INC.

TIME PERIOD	ANALYSIS OF VARIANCE			BARTLETT'S TEST			EXPERIMENTAL GROUPS (MEAN AND SIGMA)						
	F-RATIO	F-STAT.	SIG. DIFF. AND CONF. LEVEL	BARTLETT RATIO	BARTLETT STAT.	SIG. DIFF. AND CONF. LEVEL	MEAN AND SIGMAS	NEG. CONTROL	POS. CONTROL	1/100 LD 50	INT. 1	INT. 2	INT. 3
Implants	1.71	2.33	No	24.67	15.1	Yes @.01	Mean 11.2 2 1.9	11.8 1.4	10.5 2.0	11.3 1.4	12.5 1.9	11.1 3.1	
Chi-Square Test Results: No significant difference at .05 confidence level.													
Corpora Lutea	15.18	3.25	Yes @.01	15.35	15.1	Yes @.01	Mean 12.1 2 2.1	12.5 1.7	18.9 4.1	16.2 2.6	14.9 2.2	14.1 2.1	
Chi-Square Test Results: 1/100 LD 50 group significantly higher than other groups at .01 confidence level.													
Viable Fetuses	1.74	2.33	No	22.59	15.1	Yes @.01	Mean 9.7 2 2.2	10.6 1.7	9.7 2.5	10.8 1.5	11.5 1.3	10.1 3.1	
Chi-Square Test Results: No significant difference at .05 confidence level.													
Non-Viable Fetuses	-	-	-	-	-	-	Mean 0.23 2 -	0.59 -	0.06 -	0.12 -	0 0	0 0	
Resorptions	2.22	2.33	No	6.97	11.1	No	Mean 1.29 2 1.10	0.59 0.80	0.73 0.96	0.35 0.61	1.00 1.07	0 0	
Chi-Square Test Results: Negative control group significantly higher than other 5 groups at .05 confidence level.													
Average Fetal Weight (gms)	0.98	2.33	No	203.1	15.1	Yes @.01	Mean 1.05 2 0.20	1.01 0.16	1.01 0.07	1.50 1.94	1.01 0.25	0 0	
Wilcoxon's Test Results: No significant difference at .05 confidence level.													

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